

AMENDMENT TO THE CLAIMS

Please amend the claims as follows.

This listing of claims will replace all prior versions, and listing, of claims in the application.

Listing of Claims:

Claim 1 (currently amended): An isolated, a synthetic, or a recombinant nucleic acid comprising:

(a) a nucleic acid sequence having at least 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:7, wherein the nucleic acid encodes ~~at least one~~ a polypeptide having an ~~amylase~~ glucoamylase activity;

~~(b) the nucleic acid of (a), wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection;~~

~~(c) the nucleic acid of (b), wherein the sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d "nr-pataa" -F F, and all other options are set to default;~~

~~[[d]]~~ (b) a nucleic acid sequence encoding a polypeptide ~~[[of]]~~ at least 95% identical to SEQ ID NO:8, ~~or an enzymatically active fragment thereof~~, wherein ~~[[in]]~~ the polypeptide ~~or fragment thereof~~ has glucoamylase activity;

~~(e) a nucleic acid sequence that hybridizes under stringent conditions to a nucleic acid comprising the sequence of SEQ ID NO:7,~~

~~wherein the stringent conditions include a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes, and nucleic acid sequence has at least 90% sequence identity to SEQ ID NO:7, and the nucleic acid encodes a polypeptide having an amylase activity; or~~

~~[[f]]~~ (c) a nucleic acid sequence encoding ~~enzymatically active~~ a fragment~~[[s]]~~ of the polypeptide ~~of (a) or (b)~~, wherein the fragment has glucoamylase activity; ~~having an amylase activity encoded by the nucleic acid of (a);~~

~~(g) the nucleic acid of any of (a) to (f), wherein the nucleic acid encodes a polypeptide having an activity comprising: hydrolyzing glucosidic bonds; a glucoamylase activity; a 1,4 α -D-glucan glucohydrolase activity; an α -amylase activity; an exoamylase activity; a β -amylase activity; hydrolyzing an α -1,4-glucosidic bond; hydrolyzing an α -1,6-glucosidic bond; hydrolyzing glucosidic bonds in a polysaccharide; hydrolyzing glucosidic bonds in the polysaccharide to produce maltodextrines; cleaving a maltose or a D-glucose unit from non-reducing end of the polysaccharide;~~

[[~~(h)~~]] (d) the nucleic acid of any of (a) to ~~(g)~~(c), wherein the nucleic acid encodes ~~[[a]]~~ the polypeptide lacking a signal (leader) sequence; or

~~(i) the nucleic acid of any of (a) to (h), wherein the nucleic acid further comprises sequence encoding a heterologous polypeptide sequence;~~

~~(j) the nucleic acid of (i), wherein the heterologous polypeptide sequence comprises a heterologous signal (leader) sequence; or~~

[[~~(k)~~]] (e) a nucleic acid sequence fully complementary to any of (a) to ~~[[~~(j)~~]]~~ (d).

Claims 2 to 45 (canceled)

Claim 46 (currently amended): A nucleic acid probe for identifying a nucleic acid encoding a polypeptide with ~~an amylase~~ glucoamylase activity, wherein the probe comprises at least 70 consecutive bases of the nucleic acid sequence of claim 1; wherein the probe identifies the nucleic acid by binding or hybridization.

Claims 47 to 55 (canceled)

Claim 56 (previously presented): An expression cassette comprising the nucleic acid sequence of claim 1.

Claim 57 (previously presented): A vector comprising the nucleic acid sequence of claim 1.

Claim 58 (currently amended): A cloning vehicle comprising:

(a) ~~the~~ nucleic acid of claim 1, or ~~[[a]] the~~ vector as set forth in claim 57, ~~wherein the cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome;~~

~~—— (b) the cloning vehicle of (a), wherein the viral vector comprises an adenovirus vector, a retroviral vector, or an adeno-associated viral vector; or~~

~~—— (c) the cloning vehicle of (a), wherein the cloning vehicle comprises a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).~~

Claims 59 to 60 (canceled)

Claim 61 (currently amended): An isolated ~~transformed~~ cell comprising:

(a) the vector of claim 57, or the nucleic acid of claim 1, ~~or~~

(b) ~~the transformed cell of (a), wherein the cell is a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell, or a plant cell.~~

Claims 62 to 72 (canceled)

Claim 73 (withdrawn – currently amended): An isolated, a synthetic, or a recombinant polypeptide comprising:

(a) an amino acid sequence encoding a polypeptide having ~~an amylase~~ a glucoamylase activity having at least ~~90%~~, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:8, ~~or~~

(b) a polypeptide encoded by the nucleic acid of claim 1, wherein the polypeptide has ~~an amylase~~ glucoamylase activity;

(c) a polypeptide ~~comprising enzymatically active fragment~~ comprising enzymatically active fragment ~~[[s]] of the polypeptide of (a) or (b), wherein the polypeptide fragment has a glucoamylase activity;~~

(d) ~~the polypeptide of (a) or (c), wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection;~~

(e) ~~the polypeptide of any of (a) to (d), having an amylase activity comprising hydrolyzing glucosidic bonds; a glucoamylase activity; a 1,4 α -D-glucan glucohydrolase~~

~~activity; an α -amylase activity; an exoamylase activity; a β -amylase activity; hydrolyzing an α -1,4-glucosidic bond; hydrolyzing an α -1,6-glucosidic bond; hydrolyzing glucosidic bonds in a polysaccharide; hydrolyzing glucosidic bonds in the polysaccharide to produce maltodextrines; cleaving a maltose or a D-glucose unit from non-reducing end of the polysaccharide;~~

[[f)] (d) the polypeptide of any of (a) to [(e)] (c), wherein the polypeptide comprises at least one glycosylation site; or

[(g)] (e) the polypeptide of any of (a) to [(f)] (d), wherein the polypeptide lacks a signal (leader) sequence[;].

~~(h) the polypeptide of any of (a) to (g), comprising a heterologous polypeptide sequence;~~
~~or~~

~~(i) the polypeptide of (h), wherein the heterologous polypeptide sequence comprises a heterologous signal (leader) sequence.~~

Claims 74 to 124 (canceled)

Claim 125 (withdrawn – currently amended): A protein preparation comprising the polypeptide of claim 73, or a polypeptide encoded by the nucleic acid of claim 1 or claim 275, wherein the protein preparation comprises a liquid, a solid, or a gel.

Claim 126 (withdrawn – currently amended): A heterodimer comprising:

(a) the polypeptide of claim 73, or a polypeptide encoded by the nucleic acid of claim 1 or claim 275, and a second domain, or

(b) the heterodimer of (a), wherein the second domain is a polypeptide and the heterodimer is a fusion protein, or the second domain is an epitope, or a tag.

Claims 127 to 129 (canceled)

Claim 130 (withdrawn – currently amended): A homodimer comprising the polypeptide of claim 73, or a polypeptide encoded by the nucleic acid of claim 1 or claim 275.

Claim 131 (withdrawn – currently amended): An immobilized polypeptide comprising:

~~(a) the polypeptide of claim 73, or a polypeptide encoded by the nucleic acid of claim 1 or claim 275, or the heterodimer claim 126, or~~

~~(b) the immobilized polypeptide of (a), wherein the polypeptide is immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphite particle, a bead, a gel, a plate, an array, or a capillary tube.~~

Claim 132 (canceled)

Claim 133 (currently amended): An array comprising:

(a) the immobilized polypeptide of claim ~~[[73]]~~ 131; ~~or the heterodimer of claim 126,~~

(b) an immobilized nucleic acid comprising the nucleic acid of claim 1, or

(c) the antibody of claim 135, or

(d) a combination thereof.

Claim 134 (canceled)

Claim 135 (withdrawn – currently amended): An isolated or recombinant antibody

(a) that specifically binds to the polypeptide of claim 73 or to a polypeptide encoded by the nucleic acid of claim 1 or claim 275, ~~or~~

~~(b) the antibody of (a), wherein the antibody is a monoclonal or a polyclonal antibody.~~

Claim 136 (canceled)

Claim 137 (withdrawn – currently amended): A hybridoma comprising an antibody that specifically binds to the polypeptide of claim 73 or to a polypeptide encoded by the nucleic acid of claim 1 or claim 275.

Claim 138 (withdrawn – currently amended): A food, feed, food supplement or feed supplement for an animal comprising:

~~(a) the polypeptide of claim 73, or a polypeptide encoded by the nucleic acid of claim 1 or claim 275~~

~~(b) the food, feed, food supplement or feed supplement of (a), wherein the polypeptide is glycosylated.~~

Claim 139 (canceled)

Claim 140 (withdrawn – previously presented): An edible enzyme delivery matrix comprising:

(a) the polypeptide of claim 73, or a polypeptide encoded by the nucleic acid of claim 1 or claim 275,

(b) the edible enzyme delivery matrix of (a), wherein the delivery matrix comprises a pellet, or the polypeptide is glycosylated, or the amylase activity is thermotolerant or thermostable.

Claims 141 to 168 (canceled)

Claim 169 (withdrawn – currently amended): A method for isolating or recovering a nucleic acid encoding a polypeptide with ~~an amylase~~ a glucoamylase activity from an environmental sample comprising:

- (A) (a) providing a polynucleotide probe comprising the nucleic acid sequence of claim 1, or a subsequence thereof;
- (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a);
- (c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and
- (d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide with ~~an amylase~~ a glucoamylase activity from an environmental sample, or

(B) the method of (A), wherein the environmental sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample, or

(C) the method of (B), wherein the biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.

Claim 170 (canceled)

Claim 171 (withdrawn – currently amended): A method of generating a variant of a nucleic acid encoding a polypeptide with ~~an amylase~~ a glucoamylase activity comprising:

(A) (a) providing a template nucleic acid comprising the nucleic acid sequence of claim 1; and

(b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid, or

(B) the method of (A), wherein the method further comprises expressing the variant nucleic acid to generate a variant amylase polypeptide, or the modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturation mutagenesis (GSSM), synthetic ligation reassembly (SLR), recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof, or

(C) the method of (A), wherein the method is iteratively repeated until ~~an amylase~~ a glucoamylase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced, or

(D) the method of (C), wherein the variant glucoamylase polypeptide is thermotolerant and retains some activity after being exposed to an elevated temperature, or the variant

glucoamylase polypeptide has increased glycosylation as compared to the glucoamylase encoded by a template nucleic acid, or

(E) the method of (C), wherein the variant glucoamylase polypeptide has ~~an amylase a~~ glucoamylase activity under a high temperature, wherein the glucoamylase encoded by the template nucleic acid is not active under the high temperature, or

(F) the method of (C), wherein the method is iteratively repeated until ~~an amylase a~~ glucoamylase coding sequence having an altered codon usage from that of the template nucleic acid is produced, or

(G) the method of (C), wherein the method is iteratively repeated until ~~an amylase a~~ glucoamylase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.

Claims 172 to 217 (canceled)

Claim 218 (withdrawn – currently amended): A method for hydrolyzing a polysaccharide comprising:

- (A) (a) providing the polypeptide of claim 73; or a polypeptide encoded by the nucleic acid of claim 1 or claim 275;
- (b) providing a composition comprising a polysaccharide; and
- (c) contacting the polypeptide of ~~step~~-(a) with the composition of ~~step~~-(b) under conditions wherein the polypeptide hydrolyzes the starch, or
- (B) the method of (A), wherein the composition comprises an α -1,4-glucosidic bond, or
- (C) the method of (A), wherein the composition comprises an α -1,6-glucosidic bond.

Claims 219 to 220 (canceled)

Claim 221 (withdrawn – currently amended): A method for liquefying or removing a polysaccharide from a composition comprising ~~the following steps~~:

- (A) (a) providing the polypeptide of claim 73; or a polypeptide encoded by the nucleic acid of claim 1 or claim 275;
- (b) providing a composition comprising a polysaccharide; and

- (c) contacting the polypeptide of ~~step~~-(a) with the composition of ~~step~~-(b) under conditions wherein the polypeptide removes or liquefies the polysaccharide; or
(B) the method of (A), wherein the composition comprises an α -1,4-glucosidic bond, or
(C) the method of (A), wherein the composition comprises an α -1,6-glucosidic bond.

Claims 222 to 224 (canceled)

Claim 225 (withdrawn – currently amended): A detergent composition comprising:

- (A) the polypeptide of claim 73, a polypeptide encoded by the nucleic acid of claim 1 or claim 275, ~~or~~
~~(B) the composition of (A), wherein the amylase is a nonsurface active amylase,~~
~~or~~
~~(C) the composition of (A), wherein the amylase is a surface active amylase.~~

Claims 226 to 228 (canceled)

Claim 229 (withdrawn – currently amended): A method for hydrolyzing a polysaccharide in a feed or a food prior to consumption by an animal comprising ~~the following steps:~~

- (A) (a) obtaining a feed or a food material comprising a polysaccharide,
(b) providing the polypeptide of claim 73; or a polypeptide encoded by the nucleic acid of claim 1 or claim 275; and
(c) adding the polypeptide of ~~step~~-(b) to the feed or food material of ~~step~~ (a) in an amount sufficient for a sufficient time period to cause hydrolysis of the polysaccharide and formation of a treated food or feed, thereby hydrolyzing the polysaccharide in the food or the feed prior to consumption by the animal, or
(B) the method of (A), wherein the food or feed comprises rice, corn, barley, wheat, legumes, or potato.

Claim 230 (canceled)

Claim 231 (withdrawn - currently amended): A method for textile processing or desizing comprising ~~the following steps~~:

- (a) providing the polypeptide of claim 73; or a polypeptide encoded by the nucleic acid of claim 1 or claim 275;
- (b) providing a textile; and
- (c) contacting the polypeptide of ~~step~~-(a) and the textile of ~~step~~-(b) under conditions wherein the amylase can process or desize the textile.

Claim 232 (withdrawn – currently amended): A method for paper, fiber or pulp processing or deinking comprising ~~the following steps~~:

- (a) providing the polypeptide of claim 73; or a polypeptide encoded by the nucleic acid of claim 1 or claim 275;
- (b) providing a composition comprising paper, pulp or fiber; and
- (c) contacting the polypeptide of ~~step~~-(a) and the composition of ~~step~~-(b) under conditions wherein the polypeptide can process or deink the paper, pulp or fiber.

Claim 233 (withdrawn – currently amended): A method for treatment of a lignocellulosic material comprising ~~the following steps~~:

- (a) providing the polypeptide of claim 73; or a polypeptide encoded by the nucleic acid of claim 1 or claim 275;
- (b) providing a lignocellulosic material; and
- (c) contacting the polypeptide of ~~step~~-(a) and the fiber of ~~step~~-(b) under conditions wherein the polypeptide can treat the material thereby improving the material properties.

Claim 234 (withdrawn – currently amended): A method for producing a high-maltose or a high-glucose syrup comprising ~~the following steps~~:

- (a) providing the polypeptide of claim 73; or a polypeptide encoded by the nucleic acid of claim 1 or claim 275;
- (b) providing a composition comprising a polysaccharide; and
- (c) contacting the polypeptide of ~~step~~-(a) and the ~~fabrie~~ composition of ~~step~~-(b) under conditions wherein the polypeptide of step (a) can hydrolyze the composition of step (b),

thereby producing a high-maltose or a high-glucose syrup, wherein the polysaccharide is from rice, corn, barley, wheat, legumes, potato, or sweet potato.

Claim 235 (canceled)

Claim 236 (withdrawn – currently amended): A method for improving the flow of a polysaccharide containing production fluid, comprising ~~the following steps~~:

- (a) providing the polypeptide of claim 73; or a polypeptide encoded by the nucleic acid of claim 1 or claim 275;
- (b) providing a production fluid comprising a polysaccharide; and
- (c) contacting the polypeptide of ~~step~~ (a) and the production fluid of ~~step~~ (b) under conditions wherein the ~~amylase~~ polypeptide can hydrolyze the polysaccharide in the production fluid, thereby improving its flow by decreasing its density; or
- (d) the method of (a) to (c), wherein the production fluid is from a subterranean formation.

Claim 237 to 240 (canceled)

Claim 241 (withdrawn – currently amended): A method for brewing or alcohol production comprising ~~the following steps~~:

- (a) providing the polypeptide of claim 73; or a polypeptide encoded by the nucleic acid of claim 1 or claim 275;
- (b) providing a composition used for brewing or in alcohol production comprising a polysaccharide;
- (c) combining the polypeptide of ~~step~~ (a) with the composition of ~~the step~~ (b) under conditions wherein the polypeptide can hydrolyze the polysaccharide in the composition used for brewing or alcohol production.

Claims 242 to 270 (canceled)

Claim 271 (withdrawn – currently amended): A method for producing a food or feed comprising:

- (a) providing the polypeptide of claim 73 or a polypeptide encoded by the nucleic acid of claim 1 or claim 275;
- (b) providing a composition comprising a food or feed; and
- (c) ~~expressing the nucleic acid to produce a recombinant amylase; and~~
- (~~d~~) mixing the ~~recombinant amylase~~ polypeptide of (a) and the ~~feed comprising composition of (b)~~, thereby producing a food or feed ~~comprising a recombinant amylase~~.

Claim 272 (withdrawn – currently amended): A milling process comprising:

- (A) use of the polypeptide of claim 73 or a polypeptide encoded by the nucleic acid of claim 1 or claim 275,
- (B) the method of (A), wherein the process further comprises use of a second polypeptide having amylase activity, an alpha amylase activity, or a beta amylase, or another enzyme,
- (C) the method of (A) or (B), wherein the milling process is a corn wet milling process,
- or
- (D) the method of (A) or (B), wherein the milling process is a dry milling process.

Claim 273 (withdrawn – currently amended): A baking process comprising:

- (A) use of the polypeptide of claim 73 or a polypeptide encoded by the nucleic acid of claim 1 or claim 275, or
- (B) the method of (A), wherein the baking process further comprises use of a second polypeptide having an amylase activity, an alpha amylase activity, or a beta amylase, or another enzyme.

Claim 274 (previously presented – currently amended): A drilling process comprising the following steps:

- (A) use of the polypeptide of claim 73 or a polypeptide encoded by the nucleic acid of claim 1 or claim 275, or

(B) the method of (A), wherein the ~~baking~~ drilling process further comprises use of a second polypeptide having an amylase activity, an alpha amylase activity, or a beta amylase, or another enzyme.

Claim 275 (new) An isolated, a synthetic or a recombinant nucleic acid sequence that hybridizes under stringent conditions to the complement of a nucleic acid comprising the sequence of SEQ ID NO:7,

wherein the stringent conditions include a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes, and the nucleic acid sequence has at least 95% sequence identity to SEQ ID NO:7, and the nucleic acid encodes a polypeptide having a glucoamylase activity.